Exhibit 2

Tsitologiia. 1975 Nov;17(11):1278-82.

Denaturation time of actomyosin exposed to different chemicals

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The frog skeleton muscle actomyosin denaturation time dependence on the concentration of salts (NaCl and CaCl2) and organic chemicals (carbohydrates, narcotics and alcohols) was investigated. The following effects were detected: phase change in denaturation time associated with the rise of concentration of chemicals under study; actomyosin stabilization effect; coincidence of concentrations giving rise to protein stability with those increasing the survival time of isolated frog sceleton muscles in vitro (literature data); denaturation effect of alcohols used, both in high and very low concentrations.

Crit Rev Food Sci Nutr. 1981;14(3):201-30.

Modification of technological properties of fish protein concentrates.

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Fish protein concentrates are mixtures of cross-linked and aggregated molecules of different muscle proteins. The final conformation of the components of the mixtures is formed as a result of procedures applied to convert the raw materials into a product of desirable and stable sensory properties, containing less than 0.1% of lipids. To achieve this end usually extraction with hot organic solvents, mainly isopropyl alcohol and 1,2-dichloroethene, followed by air drying are employed. These conditions bring about denaturation of many of the proteins followed by aggregation of the molecules due to the interaction of reactive functional groups in extended polypeptide chains. In the final product a large proportion of hydrophobic groups is exposed to the solvent and the proteins exhibit an extremely low water affinity. Such concentrates, although valuable as protein supplements, have only limited suitability as active components of various processed foods, as they have poor technological value. They are insoluble or have a very low water dispersibility and swelling ability, do not form gels after heating, or have any significant fat-emulsifying capacity. Changing the dissociation or number of ionic groups of the molecules prior to extraction, e.g., by acidifying or acylating, can partially reduce the denaturing effect of heat and organic solvents and thus improve the functional properties of the product. An upgrading of the quality of concentrates produced by hot extraction can be achieved by partial enzymatic or chemical deaggregation, hydrolysis followed by the plastein reaction, or formation of suitable derivatives. The best results have been obtained by partial hydrolysis of acylated proteins or precipitation of the aggregated products using sodium hexametaphosphate. The functional properties of such products are comparable to those of vegetable protein isolates used as meat extenders. Various proteins of high technological value can be also obtained by enzymatic hydrolysis of the raw material, followed by separation of the lipids without organic solvent extraction. Such products, however, have a distinct odor and flavor and must be stabilized because of residual lipids.

J Chromatogr. 1984 Dec 28;317:227-43.

Kinetics of unfolding of proteins on hydrophobic surfaces in reversed-phase liquid chromatography.

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As a continuation of previous studies, we present in this paper measurements on the kinetics of denaturation of papain, soybean trypsin inhibitor and lysozyme on n-butyl-bonded silica gel surfaces used in reversed-phase liquid chromatography (RPLC). In all cases, native and denatured peaks widely separated from one another are observed. The rate constants for denaturation or unfolding are determined by the measurement of the peak area of the native protein as a function of the incubation time that the species spends on the bonded-phase surface. The results reveal that a slow denaturation step occurs with a half-life of ca. 15 min. In addition, studies of denaturation as a function of the amount of 1-propanol in the initial mobile phase suggest an additional unfolding step when the protein comes in contact with the bonded-phase surface. The extent of this latter step decreases as the concentration of 1-propanol increases, further suggesting that 1-propanol sorption on the bonded stationary phase may play a role in this behavior. Other studies are conducted with alpha-chymotrypsinogen, in which injection is made after the start of the gradient. The extent of denaturation is observed to be a function of the organic modifier employed. The results of this paper provide insight into the denaturation process in RPLC and suggest approaches to minimize this behavior.

Eur J Biochem. 1991 May 23;198(1):31-41.

Denaturation capacity: a new quantitative criterion for selection of organic solvents as reaction media in biocatalysis.

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The process of reversible denaturation of several proteins (alpha-chymotrypsin, trypsin, laccase, chymotrypsinogen, cytochrome c and myoglobin) by a broad series of organic solvents of different nature was investigated using both our own and literature data, based on the results of kinetic and spectroscopic measurements. In all systems studied, the denaturation proceeded in a threshold manner, i.e. an abrupt change in catalytic and/or spectroscopic properties of dissolved proteins was observed after a certain threshold concentration of the organic solvent had been reached. To account for the observed features of the denaturation process, a thermodynamic model of the reversible protein denaturation by organic solvents was developed, based on the widely accepted notion that

an undisturbed water shell around the protein globule is a prerequisite for the retention of the native state of the protein. The quantitative treatment led to the equation relating the threshold concentration of the organic solvent with its physicochemical characteristics, such as hydrophobicity, solvating ability and molecular geometry. This equation described well the experimental data for all proteins tested. Based on the thermodynamic model of protein denaturation, a novel quantitative parameter characterizing the denaturing strength of organic solvents, called the denaturation capacity (DC), was suggested. Different organic solvents, arranged according to their DC values, form the DC scale of organic solvents which permits theoretical prediction of the threshold concentration of any organic solvent for a given protein. The validity of the DC scale for this kind of prediction was verified for all proteins tested and a large number of organic solvents.

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The effect of water content and nature of organic solvent on enzyme activity in low-water media. A quantitative description

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A simple theoretical model was suggested to describe quantitatively the effect of water content and nature of organic solvents on catalytic behavior of enzymes suspended in low-water media. The model was based on a generally accepted notion that the destruction of the protein hydration shell is one of the main reasons for protein denaturation by organic solvents. The validity of the model was confirmed by the example of catalytic behavior of immobilized laccase suspended in water/organic mixtures of different compositions. In addition, the results were used to demonstrate that the effect of organic solvents and/or water content on catalytic behavior of enzymes in low-water media can be adequately assessed only in terms of the full kinetic description based on properly determined Vm and Km values.

Process for the isolation and purification of isoflavones

United States Patent 5679806 (Filed 02/24/1995, granted 10/21/1997)

Abstract: The present invention relates to a process for the isolation and purification of isoflavones from a number of different biomass sources. More particularly, the present invention relates to a three-step process whereby a biomass containing isoflavones with a solvent thereby forming an extract that is subsequently fractionated using a reverse phase matrix in combination with a step gradient elution, wherein the resulting fractions eluted from the column contain specific isoflavones that are later crystallized. The purified isoflavone glycosides may then be hydrolyzed to their respective aglycone.